REGENERATION OF PROPORTIONATE STRUCTURES IN STENTOR.

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THE important results of Gruber and of Balbiani on the power of regeneration of pieces of *Stentor coeruleus* opened the way for further experiments; and the works of Johnson and of Lillie on the same form have added some further results of interest. There remained, however, one problem that had not been touched upon by these investigators, an answer to which is needed to make more complete our knowledge of the regeneration of unicellular forms. I refer to the question of the proportionate development of the new organs in pieces of different sizes, and from different parts of the body; and also the no less important question of the change in size of old organs that may be present on the piece at the time of its removal. It is the purpose of the present communication to describe certain experiments that bear on these questions.

Although it is evident, in a general way, from the figures given by Gruber and by Balbiani that a small piece produces a smaller peristome than does a large piece, yet their figures do not show definitely that such is the case, and, in fact, it would be difficult to determine that such is the case from observations made on the swimming animals. The figures that have so far been published represent the new stentor as it appears while contracted or when swimming. To obtain sufficiently accurate data for the problems that I wished to examine, it was necessary to make the measurements and drawings from the stentor at rest when in a fully expanded condition. The object of my work was to find an answer to the following questions: 1. Do small pieces produce a new organism having the typical proportions of the normal; and does it make any difference in this respect as to the part of the stentor from which the piece is taken? 2. If a piece containing the old

peristome is cut off, will it retain the old peristome, or absorb it and produce a new one of proportionate size? If the old peristome persists, will it decrease in size until it has assumed the typical proportions? 3. If a part only of the old peristome is left on a piece, will the missing parts be regenerated from it, or will a new peristome develop?

There appeared during January and February in one of the aquaria in the laboratory a large number of stentors, whose presence seemed to be connected with the appearance of vast numbers of vorticellas, on which they fed. The operation of cutting the stentor in two or more parts was carried out either by means of small scissors, or, in most cases, by a sharp scalpel. The latter operation is greatly facilitated by placing the animals in a dish of water, the bottom of which is covered by a layer of paraffin.

The following measurements give the length of the normal blue stentor and the greatest width of the peristome.

Length.	Width of Peristome.
2.8 mm.	.52 mm.
1.6	.46
1.4	.40
1.7	.50
1.7	.48
1.6	.44
1.9	.48

If a stentor is cut in two by a cross-cut, as indicated in Fig. 1, *A*, *a–a* (the anterior piece, *B*, being smaller than the posterior, *C*), the cut surfaces of each piece are closed almost instantly by the outer layer bending over the exposed part. Only a faint, clear line on the surface indicates where the cut has been made. The history of the anterior piece is as follows: In the course of an hour or two the piece becomes somewhat more pointed at the posterior end, and then fixes itself by a foot that appears at that end (Fig. 1, *B*). The posterior end now begins to draw out into a stalk, and after thirteen hours the piece has assumed the form shown in Fig. 1, B^1. The piece is still proportionately too broad for its length, for although the peristome has become reduced in size it is not as

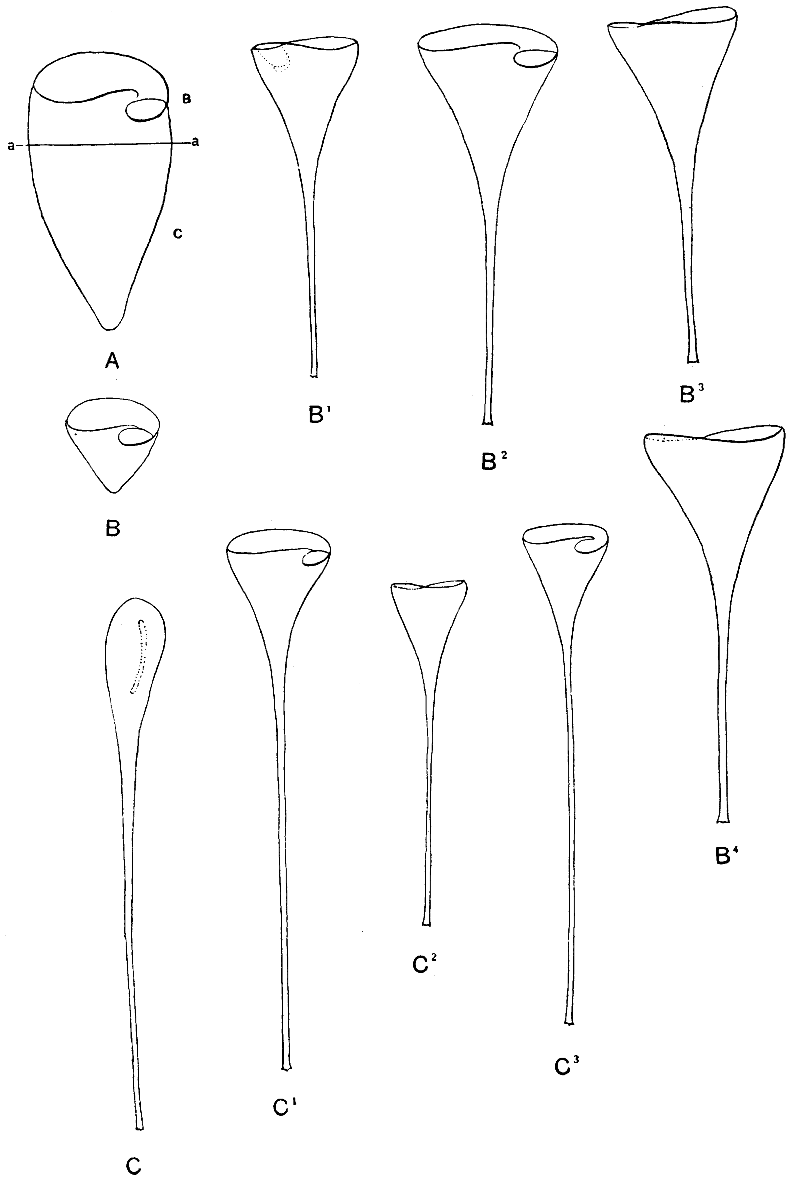

FIG. 1.—*A*, contracted stentor to show where the cut was made, *a–a*; *B*, anterior end five hours after the operation; B^1, an anterior piece thirteen hours after operation; B^2, B^3, anterior pieces twenty-four hours after operation; B^4, anterior piece forty-eight hours after the operation.

C, posterior or foot end of *A*; C^1, posterior end five hours after operation; C^2, C^3, posterior ends twenty-four hours after operation.

yet reduced sufficiently to give the piece the typical proportions. Two other head-pieces of this same series are shown in Fig. 1, B^2, B^3, that were drawn twenty-four hours after the operation. It is even more evident in these (compare with Fig. 2, *A*, for normal) than in the last that the peristome is too broad for the length of the stentor. Even after another twenty-four hours one of the pieces had still retained the same form as shown in Fig. 1, B^4.

The posterior piece, *C*, fixes itself at once by the old foot, and may soon elongate to its full length. In the course of two or three hours a clear band appears extending somewhat obliquely over the rounded end of the piece (Fig. 1, *C*). Cilia appear along the band. In a few more hours, the rate depending on the temperature, the ciliated band moves forward around the anterior end of the piece, and in doing so bends around on itself into the characteristic peristome. A new peristome-field, or disk marked by delicate parallel lines, appears on the inner side of the band even before it moves forward, and as the band bends around to make the terminal peristome, the new disk comes to lie in its central part. A depression, that appears at the basal end of the band, forms the pharyngeal funnel. The new peristome is smaller than that of the original animal, and, as the figures show (Fig. 1, C^1, C^2, C^3), it is, in some cases, even smaller than the reduced peristome on the anterior piece. The foot-piece is also at first very long as compared with the size of the new peristome ; and this condition may remain for several days.

These results demonstrate that, for some time after the new organs have developed, the new stentors retain some of the peculiarities of the part of body from which they have come. When the pieces are contracted, or are swimming, these relations are scarcely evident and might easily escape detection.

The transformation of an anterior piece into a new stentor is much more strikingly seen when only a small part of the anterior end is cut off. One set of observations on the same individual is represented in Fig. 2. The stentor fully extended is represented in Fig. 2, *A*. The anterior end had been cut off, as shown in Fig. 2, A^1, while the animal was contracted.

After its removal the anterior end, *B*, contracted still more, so that its posterior cut-surface was quickly covered over by the bending in of the sides; the disk bulged forward. After a few hours the piece became somewhat pointed at its posterior end, and then fixed itself by a foot that appeared at the end.

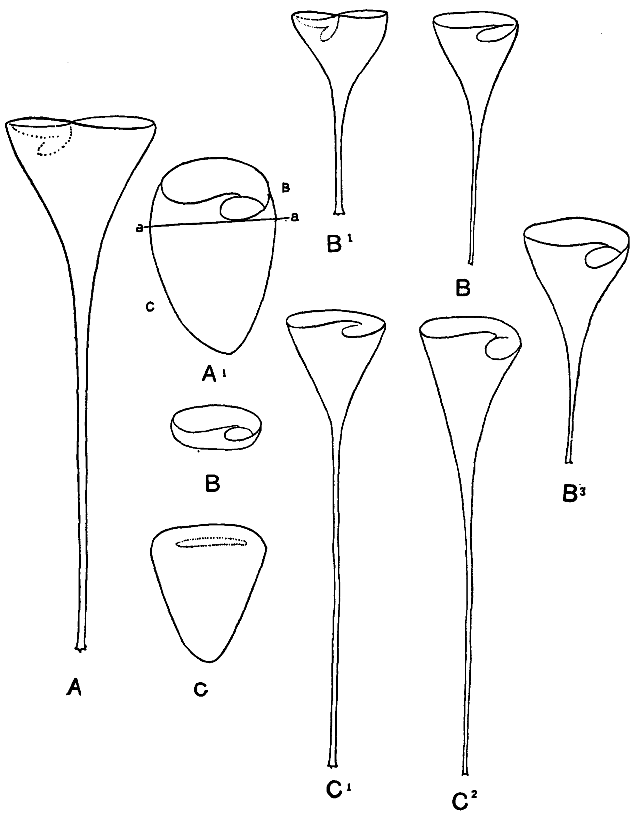

FIG. 2.—*A*, 1.4 × .4 mm. Stentor fully extended; *A*[1], same contracted, cut in two at *a–a*; *B*, *C*, immediately after operation; *B*[1], .56 × .26, anterior end after twenty-four hours; *B*[2], .7 × .25, same after another forty-eight hours; *B*[3], .64 × .28, same after another four days, *i.e.*, seven days after operation; *C*, 1.2 × .27, posterior end after twenty-four hours; *C*[2], 1.2 × .20, same after another forty-eight hours.

After twenty-four hours (it had been kept in the cold overnight, *i.e.*, for ten hours) it appeared as shown in Fig. 2, *B*[1]. The old stentor measured 1.4 mm. by .4 mm. This new stentor measures .56 mm. by .26. The old peristome has, therefore, decreased nearly to half its original width. Two

days later (Fig. 2, B^2) the stalk was somewhat longer, and after another four days (Fig. 2, B^3) the form had not materially changed. The development of the posterior piece of this same individual is shown in Fig. 2, C^1 and C^2. The piece is about twice as long as the anterior piece, but its peristome is about the same size.

A similar operation was carried out on another individual; the results are shown in Fig. 3, *A*. A very small part of the

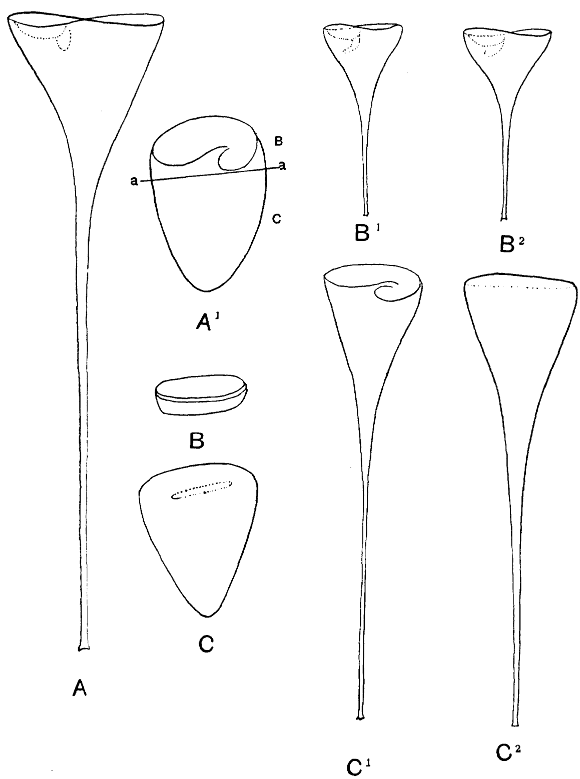

FIG. 3.—*A*, 1.7 × 4. Stentor fully extended; A^1, same contracted, cut in two at *a–a*; *B*, *C*, immediately after operation; B^1, .5 × .21, five hours after operation; B^2, .5 × .24, three days after operation; C^1, posterior piece, 1.2 × .27, five hours after operation; C^2, same, 1.6 × .32, three days after operation. In Figs. 2 and 3 one individual used in each. In Fig. 1 several individuals were used.

anterior end was cut off (Fig. 3, A^1). It contained, however, the entire peristome and disk (Fig. 3, *B*). About thirty hours after the operation the anterior piece appeared as shown in

Fig. 3, B^1. After three days more the new stentor had about the same form. The peristome is, as compared to that of the original stentor, too wide for the length of the new individual, although it is not much more than half the width of the old peristome.

The development of the posterior piece is shown in Fig. 3, C^1 and C^2. In this piece, particularly in the earlier stage, C^1, the peristome is smaller than was the original peristome, and also relatively smaller as compared with the entire length of the animal.

In order to be certain that the anterior pieces did not produce new peristomes during the night, they were kept in a cold place when not under observation; for I had found that under these circumstances the formation of a new peristome is greatly delayed, even although it may have begun to develop before the piece is subjected to the cold. In this way I could retard the development of the peristome for twelve hours, so that I felt certain that a new peristome had not developed on these anterior pieces in my absence.

In other experiments pieces of different sizes were cut from the foot-end in order to see if the size of the new peristome that is formed is in proportion to the size of the piece. It was found that a smaller peristome develops on a smaller piece, and a larger one on a larger piece; and this same relation holds also for pieces of different sizes for other parts of the body. It has been shown that cross-pieces from the anterior or posterior ends retain some of their original peculiarities even after the formation of a new individual, and that for several days the stentors from anterior pieces are too broad for their length, and individuals from the posterior end are too long for their breadth. Some of these newly regenerated stentors from the anterior pieces were kept for a longer period and supplied with food. Their measurements for from one to seventeen days after the operation are given in the following table. The measurements of three normal individuals of this lot were $1.6 \times .5$; $1.4 \times .4$; $1.1 \times .4$. The experiment began February 3.

ANTERIOR PIECES OF HALF SIZE OR LESS.

Feb. 4.	.6 × .22
	.85 × .28
	.6 × .23
Feb. 6.	.6 × .23
	.6 × .3
	.68 × .2
	.7 × .25
Feb. 10.	.85 × .32
	.76 × .28
Feb. 13.	.7 × .34
	.8 × .35 × .25
	.9 × .28 × .2
	.64 × .28
	1.12 × .39
	1.2 × .28
Feb. 20.	.96 × .36
	1.00 × .39
	1.04 × .32
	1.00 × .40
	1.00 × .40
	1.00 × .39
	.9 × .36

It will be seen from this table that, after feeding, the stentors from anterior pieces grow larger. The increase takes place both in the peristome and in the length of the piece, so that the proportionate size of the disk to the rest of the piece remains about the same. The new stentors had begun to divide on February 13, and by February 20 there were about twice as many present as at first. They have, however, about the same proportionate size as at first. The question arises whether in the normal stentor the ratio of the breadth of the disk to the length of the pieces may not be less than in very large individuals. I measured some of the smaller individuals found in the aquaria with the larger ones and obtained the following results: 1.04 × .36; .72 × .26; .7 × .28; .72 × .3; .9 × .38 × .2; 1.1 × .28; .9 × .36 × .2; .98 × .28. There is seen to be some variation in the relative size of the length to the breadth; that is due in part to the individuals not always expanding to the same extent, and also in part to some of the measurements of the peristome not having been made in the widest part, but there are also actual differences, as some very careful measurements have shown. It will be seen that while in large stentors the greatest breadth of the peristome is about one-fourth, or nearly so, of the total length, in the small individuals the breadth is more nearly one-third of the length; therefore the peristome is proportionally somewhat larger for smaller pieces. Comparing these measurements with those of the sizes of individuals derived from pieces of the anterior end, we see that they have reached in several cases the characteristic form *for a small individual.* Since there is

a good deal of variation in the proportion between the width of the peristome and the length of the animal both for small normal individuals and for those that have come from anterior pieces, it may be stated that pieces from the anterior end may produce new stentors whose proportions come within the range of variation of size of normal small stentors of about the same length. The measurements of posterior pieces, that are at first too long for the size of the peristome, show that they, too, assume more typical proportions. Thus one of the posterior ends of the last series measured, on February 6, $1.0 \times .25$. On February 10, two other individuals in the same list measured $1.4 \times .38$; $1.2 \times .4$. The peristomial region had, therefore, reached the full size.

A somewhat crude comparison may bring the results home. If a man were cut in two at the waist and the pieces behaved in the same way as those of stentor, two new individuals would develop. The anterior half would produce a small man with a head too large for his height, *i.e.*, his legs wòuld be too short for a man with that sized head. Although the old head had grown smaller, it would be still too large for the rest of the new man. In fact, his proportions would be more like those of a baby whose head is relatively too large for his length as compared with that of a man, and his legs too short. It is just this result that we have found for anterior pieces of stentor. If the new man were supplied with food, all parts of the body would grow larger; but as he got larger his legs would grow faster than his head.

The posterior end of our imaginary man would have at first legs too long for his total length, and his new head would be relatively too small; but if he were fed his head, shoulders, and arms would grow faster than any other part and continue to grow until the proportionate size had been reached. If he were not fed, it is possible that his head and upper part might increase more slowly in size at the expense of the material in his legs, and the latter would get smaller until a balance was reached. The result would be that a boy rather than a baby was produced.

In other experiments pieces were removed that contained

only a part of the peristome. In one series these pieces were cut off, as shown by the line *a–a* in Fig. 4, *A*; so that there was a smaller and a larger piece, the former, *B*, containing a part of the old peristome, but not any part of the pharyngeal funnel, and the latter, the larger piece, *C*, containing also a

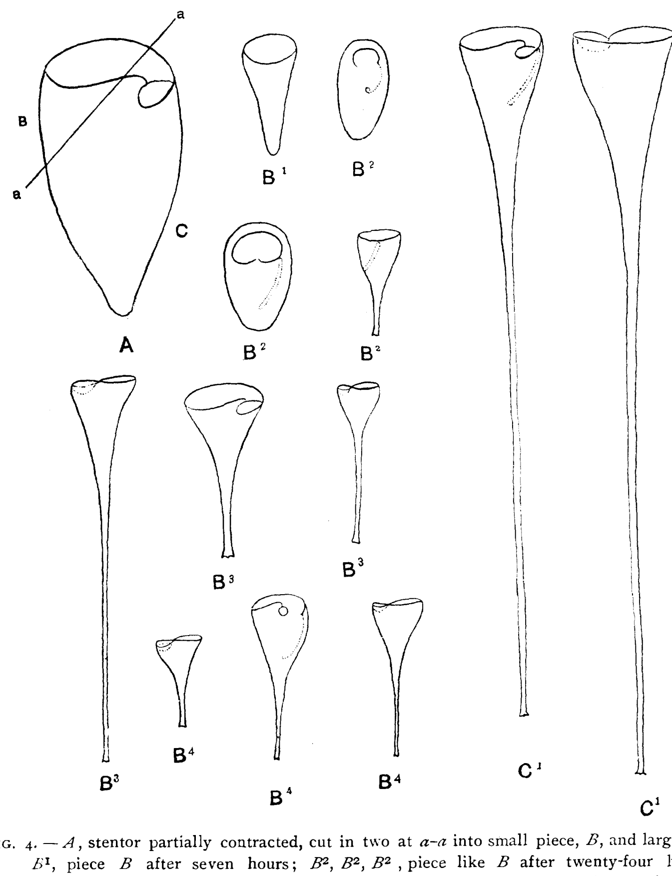

FIG. 4. — *A*, stentor partially contracted, cut in two at *a–a* into small piece, *B*, and larger, *C*: B^1, piece *B* after seven hours; B^2, B^2, B^2, piece like *B* after twenty-four hours; B^3, B^3, B^3, same after twenty-nine hours; B^4, B^4, B^4, same in part after forty-eight hours (after operation). C^1, C^1, piece *C* after seven hours.

part of the peristome as well as the funnel. The cut surface of each piece is quickly closed by the bending in of the sides, and the cut ends of the peristome are generally brought together to make a closed ring. A foot develops on the basal end of the anterior piece, *B*, B^1, and a stalk is soon produced in that region. This piece may remain for twenty-four hours in this

condition. Sooner or later a new ciliated band appears on the old wall behind the part of the old peristome, as shown in Fig. 4, B^2. The band moves forward, fusing with the old ring at one point and, replacing the latter, produces a new peristome. Whether the piece of the old ring is entirely obliterated, or whether a part of it remains to contribute to the new peristome, I did not determine. The regeneration of a new peristome on these pieces may be delayed for several days (Fig. 4, B^4), and, in general, does not appear as soon as on pieces that do not contain any part of the old peristome. Five or six series of experiments of this sort, each series of a number of pieces, were made, and the smaller pieces followed with great care. Only those smaller pieces were isolated that contained no part of the old funnel. Nearly all the pieces behaved in the way just described, but in one or two a small funnel developed where the cut edges came together. This may have been due to a very small piece of the original funnel having been cut off, or to the piece having come from very near to the old funnel, or, as seems more probable, to the development of a new funnel from the old ciliated band. If the last interpretation is correct, it shows that in exceptional cases the peristome may complete itself. In the large majority of cases this does not occur and a new peristome and funnel develop at the side and move forward. In nearly all cases the cut ends of the old peristome come together, meeting in a slight notch. In one or two instances one band lay slightly below the other at the meeting point, producing a peristome exactly like the normal in shape, only the funnel was absent. If a small piece of the old funnel is left, it assumes the characteristic position of the funnel, and, in fact, becomes such to all appearance, although this peristome is generally replaced later by a new one.

Gruber studied the regeneration of pieces somewhat similar to these without a funnel, and states that the remaining part of the old peristome gives rise to a new one, but I have not found this to be the case. If the piece is kept under close observation, the development of a new peristome is found to take place in the way just described. The change is sometimes so rapid that a few hours may suffice to bring it about.

The history of the complementary piece (Fig. 4, *C*) is as follows: After the cut surface has been closed over and the edges of the peristome brought together, the piece may immediately fix itself by the old foot. The piece elongates to its full length, which is the same as that of the former animal (Fig. 4, C^1). In some of these pieces I have observed the development of a new peristome in the course of a few hours after the operation (Fig. 4, C^1). It seems that this takes place sooner when only a small part of the old peristome and funnel is left than when a larger part remains. In cases in which a large part of the old peristome remains a new peristome may not develop for several days; and in some cases I have not found it to appear at all, but I cannot state positively that it does not ultimately appear. Since even normal individuals may produce a new peristome, the appearance of a peristome on these new stentors after several days may be only the regular process of renewal of that organ. In two cases, in which a new peristome appeared after two days, the old one had begun to break down while the new band was developing. In all other cases the old part was still active and normal in appearance up to the time of its replacement by the new ciliated band. These results show that even in a large piece the new peristome is not regenerated from the old one. The presence of the old pharyngeal funnel in these pieces does not make any important difference in the end result, although it may be that pieces of this sort regenerate less quickly than when the piece does not contain the funnel portion of the old peristome.

Another experiment that supplements the preceding one in several respects consists in cutting the stentor in two, as indicated by the line *a–a* in Fig. 5, *A*. In this case the smaller piece, *B*, contains the funnel part of the peristome, while the larger piece, *C*, contains the remaining part of the peristome.

The smaller piece, *B*, closes in, develops a foot, becomes attached and produces a stalk. The edges of the peristomial ring unite more or less, as shown in Fig. 5, B^1. In one case a new peristomial band appeared six hours after the operation, moved forward, and produced a new peristome (Fig. 5, B^1). In

other cases, in which the piece was small in comparison to the size of the remaining part of the peristome, a new peristome did not appear in one case until after thirty hours (Fig. 5, B^4); in other cases a new peristome had not appeared at this time (Fig. 5, B^3).

The complementary piece (*C*, Fig. 5) closed in, fixed itself, and extended to its full length. In pieces in which the

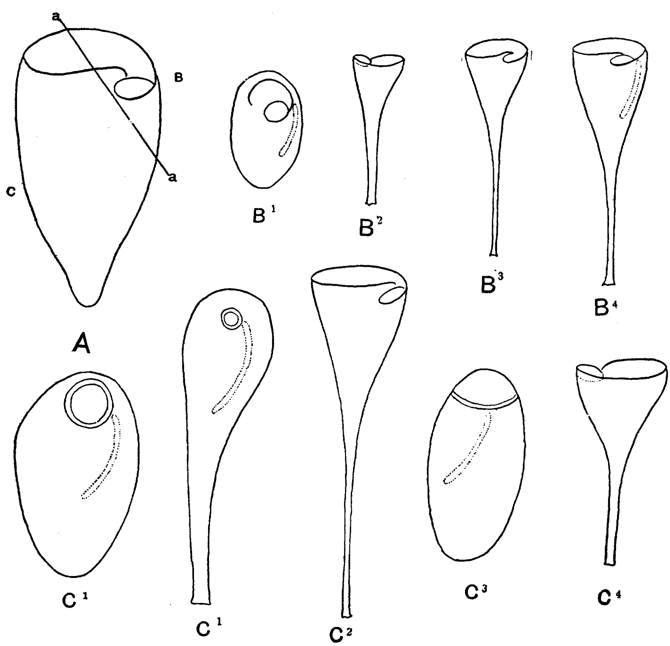

FIG. 5.—*A*, stentor partially contracted, cut in two at *a–a* into a small piece, *B*, and a large piece, *C*; B^1, piece like *B* after seven hours; B^2, piece like *B* after twenty-seven hours; B^3, B^4, piece like *B* after twenty-nine hours; C^1, C^1, piece like *C* after seven hours; C^2, piece like *C* after twenty-nine hours; C^3, C^4, piece like *C* after fifty-one hours.

remaining part of the peristome, that had united to make a ring, was quite small a new ciliated band appeared in four hours; in others, in six hours (Fig. 5, C^1 C^1); and in pieces with a larger peristomial region, after twenty-four and even after fifty-one hours (Fig. 5, C^3). It is interesting to note that in these pieces the region from which in the normal individual the peristomial band is formed has been more or less completely removed, yet a new peristomial band may very quickly

appear. I did not attempt to determine the position of the new band in its relation to the region of closure of the piece.

In several cases in the last two experiments, and in some other experiments like those shown in Fig. 6, *A*, B^1, C^1, small pieces were sometimes cut off that contained a part of the old peristome, but which did not fix themselves, or assume the characteristic form. As these pieces were generally small, although not below the minimal size, there can be little doubt that most of them did not contain any part of the nucleus, and in several cases I proved this to be the case by staining the pieces in picro-carmine. The result shows that in the absence of the nucleus a piece containing a part of the old peristome cannot complete the peristome from the remaining part. This is the

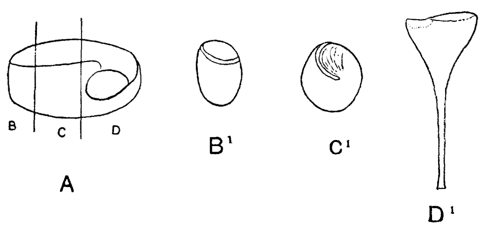

FIG. 6.—*A*, anterior end of stentor cut off and then divided into three pieces. Two of these, B^1, C^1, were apparently without nuclei, and did not produce a new peristome or assume the typical form.

less to be expected since it has been shown that even in nucleated pieces the new peristome is produced not by the old one, but by the development of a new peristomial band. The result is interesting in connection with a result obtained by Gruber, *viz.*, that if a non-nucleated piece containing a part of the newly forming ciliated band is obtained it produces from the band a new peristome. My results show that a piece of the old band cannot act in this way. That the presence of the nucleus is connected with the formation of a new peristomial band seems highly probable, but I can easily imagine that could a non-nucleated piece be supplied with certain unformed elements it might be capable of producing a new peristome. The results do not seem to me to show more than that the nucleus supplies certain products of metabolism that must be present before the protoplasm can successfully carry out its

innate tendency to complete the typical form. We are not justified, I believe, in drawing the conclusion, as Gruber has done, that preformed elements of the peristome exist in the nucleus and must be set free in order to initiate the development of a new peristome.

Lillie has found that the smallest piece of *Stentor polymorphus* that becomes a perfect form is equal to a sphere of about 80 μ in diameter. The average size of the stentors was equal to 230 μ. This makes the volume of the smallest stentor about $\frac{1}{27}$ of the normal. For *Stentor coeruleus* the smallest stentors measured 90 μ ($=\frac{1}{11}$ mm.), the average normal stentor 280 μ ($=\frac{7}{25}$ mm.). Therefore the former is about $\frac{1}{27}$ of the latter.

Although I have not worked specially on this problem, yet I have obtained some small stentors that were proportionately smaller than those obtained by Lillie. Thus one individual measured when extended .25 × – .08 mm., and when contracted into an oval or nearly into a sphere .08 × .08 ($=\frac{1}{12}$ mm.). The larger normal stentors measured about .4 × .32 × .32 when contracted. Although it is only possible to give a general estimate of the relative size of these two individuals, the smaller cannot be over $\frac{1}{64}$ of the former. It would be a mistake to infer from this, as well as from Lillie's calculations, that the latter came from a piece $\frac{1}{64}$ or even $\frac{1}{27}$ of the original stentor. The protoplasm of stentor is so vacuolated that a piece losing the fluid in the protoplasm might become much smaller than when first removed.

Lillie states that he believes that it would be possible to obtain a smaller individual of *S. coeruleus* than $\frac{1}{11}$ mm. The one that I obtained was in fact somewhat smaller, *viz.*, $\frac{1}{12}$ mm. The difference in our results depends, therefore, rather on the size of the normal average stentor with which the comparison is made than on the smallest individual obtained. Lillie says that he does not think there can be much difference in the absolute size of the smallest stentors, whether one uses the largest or the smallest normal speçimens. It seems to me that this may or may not be true, according to what factors may enter into the result.

The conclusion that a piece $\frac{1}{27}$ or even $\frac{1}{64}$ of the entire animal can produce a new individual can give only a most general idea of the relative size of the smallest piece, since more depends on the size of the normal individual than on that of the smallest pieces, and there is for stentor a very wide range of size that may be called normal. A large normal individual may contain eight times the volume of a small normal individual. More significant, therefore, is the absolute size of the smallest piece capable of regeneration, and in this respect my results are practically in accord with those of Lillie.

Several experiments were made in which pieces were cut in two longitudinally. In a longer or shorter time most of the halves produced a new peristomial band that became a new peristome. As this experiment did not give promise of much that is new, it was tried in only a few cases.

A few casual observations made during the course of the work may be briefly mentioned. The stentors were observed dividing on several occasions, but Johnson's excellent figures and account of these stages leave nothing new for me to add. I have often noticed that after division the two products are found attached side by side, and if they are not disturbed a little colony may arise in the same spot. Several times I have observed that two individuals that have been formed by division of one of the regenerated posterior pieces were unequal in size, although I do not know whether the smaller individual was the distal or the proximal one.

As Gruber has pointed out, the first steps in the process of division and of regeneration are the same, and this holds also for the physiological replacement of the old peristome. In all cases a peristomial ciliated band appears *on the side* and moves forward around the anterior end to become the new peristome. We have here another illustration that shows that during the process of regeneration the factors that appear in the normal growth may take part in the regeneration, and this relation appears to hold for unicellular as well as for multicellular forms.

In many cases, especially where a somewhat oblique cut has been made, the superficial blue stripes come together over the

cut surface in a most irregular way, yet this does not appear to interfere with the subsequent regeneration; and after a time the stripes appear to be more regularly arranged. That a certain amount of absorption takes place, and possibly also development of new stripes, seems probable, but I have not studied these changes in any detail. It would be interesting to find out if in cross-pieces of the body the number of the stripes remains the same and their size becomes smaller, or whether the number of the stripes is proportionately reduced.

On several occasions I have tried to graft together pieces of different stentors, but the exposed surfaces close so quickly that I have not been able to get the pieces to unite. It does not seem altogether improbable that the result could be brought about by cutting two stentors at the same time, one about the other. A lucky cut might bring two exposed inner surfaces together, and they might stick to each other, but so far I have not been able to carry out successfully this experiment.

In a few cases the stentor was split partially in two pieces, but generally the halves soon fuse together. It is of some interest to find that, although the peristome was cut in two and had reunited, a new peristome was not produced, showing that the operation alone does not initiate the changes that lead to the development of a new peristome.

The development of a new peristome in a piece that contains a part of the old one appears to be due to the lack of proportion between the old part (even when it contains all the essential parts of the peristome) and the rest of the piece. This result is unique, since in all other forms in which a part of an old organ remains the new organ regenerates from that part. In stentor this does not occur, but a new organ is produced. It is important to observe, however, that this is the characteristic way in which stentor produces a peristome, so that the organisms make use of a process that already exists. The reduction in size of the old peristome in pieces from the anterior end is the result that has most interested me. It seems to be due to the withdrawal of material from the anterior region to form the body and stalk of the new stentor.

The change in shape of the piece, *i.e.*, the production of the typical form, is primarily the result of a shifting of the material that carries with it a loss of material in the old part. Other so-called formative factors may have some share in the reduction in size of the old peristome to one proportionate to the rest of the piece, but the simple loss of material will, I think, account for the greater part of the change.

What primarily brings about this change in the material so that the typical form is produced is a question to which at present there is no answer.

BRYN MAWR, March 12, 1901.